

5 from viral genomes. Commonly used promoter sequences and enhancer sequences which
may be used in the present invention include, but are not limited to, those derived from human
cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40).
Methods for the construction of mammalian expression vectors are disclosed, for example, in
10 5 Okayama and Berg (*Mol. Cell. Biol.* 3:280 (1983)); Cosman *et al.* (*Mol. Immunol.* 23:935
(1986)); Cosman *et al.* (*Nature* 312:768 (1984)); EP-A-0367566, and WO 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and
monoclonal antibodies, which are useful in diagnostic assays for detecting Hu-Asp
15 polypeptide expression. Such antibodies may be prepared by conventional techniques. See,
for example, *Antibodies. A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies. Hybridomas: A
New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980).
20 Synthetic peptides comprising portions of Hu-Asp containing 5 to 20 amino acids may also be
used for the production of polyclonal or monoclonal antibodies after linkage to a suitable
25 15 carrier protein including but not limited to keyhole limpet hemacyanin (KLH), chicken
ovalbumin, or bovine serum albumin using various cross-linking reagents including
carbodiimides, glutaraldehyde, or if the peptide contains a cysteine, N-methylmaleimide. A
preferred peptide for immunization when conjugated to KLH contains the C-terminus of
30 Hu-Asp1 or Hu-Asp2 comprising QRRPRDPEVVNDESSLVRHRWK or
20 LRQQHDDFADDISLLK, respectively.

The Hu-Asp nucleic acid molecules of the present invention are also valuable for
chromosome identification, as they can hybridize with a specific location on a human
35 chromosome. Hu-Asp1 has been localized to chromosome 21, while Hu-Asp2 has been
localized to chromosome 11q23.3-24.1. There is a current need for identifying particular sites
25 25 on the chromosome, as few chromosome marking reagents based on actual sequence data
(repeat polymorphisms) are presently available for marking chromosomal location. Once a
40 sequence has been mapped to a precise chromosomal location, the physical position of the
sequence on the chromosome can be correlated with genetic map data. The relationship
45 45 between genes and diseases that have been mapped to the same chromosomal region can then
30 be identified through linkage analysis, wherein the coinherence of physically adjacent genes
is determined. Whether a gene appearing to be related to a particular disease is in fact the
cause of the disease can then be determined by comparing the nucleic acid sequence between
50 affected and unaffected individuals.

5 In another embodiment, the invention relates to a method of assaying Hu-Asp function, specifically Hu-Asp2 function which involves incubating in solution the Hu-Asp polypeptide with a suitable substrate including but not limited to a synthetic peptide containing the β -secretase cleavage site of APP, preferably one containing the mutation found in a Swedish kindred with inherited AD in which KM is changed to NL, such peptide comprising the sequence SEVNLDAEFR in an acidic buffering solution, preferably an acidic buffering solution of pH5.5 (see Example 12) using cleavage of the peptide monitored by high performance liquid chromatography as a measure of Hu-Asp proteolytic activity. Preferred assays for proteolytic activity utilize internally quenched peptide assay substrates. Such suitable substrates include peptides which have attached a paired fluorophore and quencher including but not limited to coumarin and dinitrophenol, respectively, such that cleavage of the peptide by the Hu-Asp results in increased fluorescence due to physical separation of the fluorophore and quencher. Preferred colorimetric assays of Hu-Asp proteolytic activity utilize other suitable substrates that include the P2 and P1 amino acids comprising the recognition site for cleavage linked to o-nitrophenol through an amide linkage, such that cleavage by the Hu-Asp results in an increase in optical density after altering the assay buffer to alkaline pH.

In another embodiment, the invention relates to a method for the identification of an agent that increases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

- (a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and
- (b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent,

whereby a higher level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has increased the activity of said Hu-Asp polypeptide. Such tests can be performed with Hu-Asp polypeptide in a cell free system and with cultured cells that express Hu-Asp as well as variants or isoforms thereof

In another embodiment, the invention relates to a method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide. Such tests can be performed with Hu-Asp polypeptide in a cell free system and with cultured cells that express Hu-Asp as well as variants or isoforms thereof.

In another embodiment, the invention relates to a novel cell line (HEK125.3 cells) for measuring processing of amyloid β peptide ($A\beta$) from the amyloid protein precursor (APP). The cells are stable transformants of human embryonic kidney 293 cells (HEK293) with a bicistronic vector derived from pIRES-EGFP (Clontech) containing a modified human APP cDNA, an internal ribosome entry site and an enhanced green fluorescent protein (EGFP) cDNA in the second cistron. The APP cDNA was modified by adding two lysine codons to the carboxyl terminus of the APP coding sequence. This increases processing of $A\beta$ peptide from human APP by 2-4 fold. This level of $A\beta$ peptide processing is 60 fold higher than is seen in nontransformed HEK293 cells. HEK125.3 cells will be useful for assays of compounds that inhibit $A\beta$ peptide processing. This invention also includes addition of two lysine residues to the C-terminus of other APP isoforms including the 751 and 770 amino acid isoforms, to isoforms of APP having mutations found in human AD including the Swedish KM \rightarrow NL and V717 \rightarrow F mutations, to C-terminal fragments of APP, such as those beginning with the β -secretase cleavage site, to C-terminal fragments of APP containing the β -secretase cleavage site which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion, and to C-terminal fragments of APP which have been operably linked to an N-terminal signal peptide for membrane insertion and secretion and a reporter sequence including but not limited to green fluorescent protein or alkaline phosphatase, such that β -secretase cleavage releases the reporter protein from the surface of cells expressing the polypeptide.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.